APPARATUS AND METHOD

FOR DETERMINING SUBSTANCES

CONTAINED IN A BODY FLUID

Insert

HF
9/03

5   BACKGROUND OF THE INVENTION

This application is a 371 of PCT/US00/16816, filed 6/16/2000, and claims benefit of Provisional Application No. 60/139983, filed 6/18/99.

The present invention relates generally to an assay system for biological

and nonbiological fluids. More particularly, the present invention relates to an

apparatus for separating serum or plasma in order to measure analytes within the

10   serum.

It has long been desirable to utilize devices that can be used for on-site

testing of blood products. Particularly important is the analysis of body fluids

from humans and animals to diagnose disease, monitor the course of therapy, or

determine the presence of illicit drugs. Human Biological fluids may include

15   blood, urine, saliva, feces, peritoneal fluid, synovial fluid, spinal fluid, interstitial

fluid, tears, cellular fluid, pancreatic or bile fluids. Commonly, the analytical

methods used to carry out these objects are performed on blood samples.

Clinical chemists have a preference for working with serum over plasma

and plasma over whole blood because of the clarity of the sample matrix and the

20   lack of interfering substances from the solid portion of the blood. In order to

facilitate analysis, a separation step must be carried out since the presence of

red blood cells, either intact or hemolyzed interferes with detection of the signal

generated by the chemical reaction performed by the test.

Conventionally, the separation of blood components has been carried out

25   by placing a clotted blood sample in a centrifuge and centrifuging the sample for

ten minutes at approximately 3,000 rpms. The serum obtained from this centrifuging step is then used to carry out the test, thus avoiding interference from blood solids such as red blood cells and clotting factors.

An embodiment for chemical tests called dry reagent strips was developed
5 first for urinalysis. Thereafter, various efforts to combine dry reagent strip technology in blood testing were started in the early 1950's. Notably, U.S. Patent No. 3,092,465 discloses a reagent in a bibulous carrier with a superimposed semipermeable coating to exclude the chemical and nonchemical interference from red blood cells. The device, while performing analysis on whole blood, still
10 required additional manipulations by the user, in the form of washing of excess blood after a specified time interval. Additionally, U.S. Patent Nos. 3,552,925 and 3,552,928 disclose the use of salts and amino acids to perform in-situ separation. U.S. Patent No. 4,477,575 discloses the use of a glass fiber matrix.

More recently, membranes have been employed in a variety of devices.
15 These include devices disclosed in the following United States and foreign patents and publications: U.S. Patent Nos. 4,774,192 and 5,166,051; European Published Applications EP 0408222 A1, EP 0408223 A1, EP 0407800 A2 and EP 0388782; and PCT Published Applications Nos. WO 93/22453, WO 95/16207 and WO 90/10869. The use of the various membranes disclosed in the above
20 patent documents operate on size exclusion principles, and several of these are limited by rates of capillary flow and do not completely eliminate interference from intact or hemolyzed red blood cells. Fresh red blood cells are elastic in nature and may pass through pores smaller than their nominal diameter. Hemolysis may occur on contact with some of the architectural or chemical components of

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the strips. Consequently, errors may be introduced into the measurement system.

U.S. Patent No. 5,104,619 discloses a disposable diagnostic system comprising a test card having a substantially flat body and a generally cylindrical

5   reagent pad pocket formed in a central area of the flat body. A reagent chemistry pad is disposed in the pocket and a snap fit cover is received in the pocket and arranged over the pad to retain the pad in position. The device size and configuration allows for bar code graphics to be printed on the underneath side of the device. The bar code may contain lot-specified data about the reagent

10   chemistry, and is read by the meter during device insertion. This data may further contain critical parameters for the software algorithm within the meter electronics. U.S. Patent No. 5,139,685 also discloses a separation filter assembly having a snap fit lid. In this patent, glass fibers are utilized and maintained in a compressed state under pressure.

15   Accordingly, a need exists for an integrated system for assaying, in one step, analytes in whole blood samples which are not affected by the chemical or physical interference normally caused by red blood cells and other portions of whole blood.

20   SUMMARY OF THE INVENTION

The present invention, in one form thereof, comprises a dry solid phase diagnostic test strip and system for the chemical, enzymatic, and/or immunological analysis of whole blood analytes, comprising a reflectance

25   photometer, a solid support strip, a porous detection zone member, a permeable

spreading and separation layer, a precipitation layer and an overlay sample

receiving membrane containing an agent, or agents for the exclusion of intact red

blood cells and a strip receiving platform with guides for positioning the strip

inside the reflectance photometer. The detection area membrane may contain

5   chemical, enzymatic, and/or immunological reagents that generate specific

signals in the presence of a target analyte. The agent, or agents, in contact with

the overlay membrane, prevents passage and hemolysis of red blood cells while

facilitating rapid transport and reaction of the plasma or serum portion of

introduced whole blood samples. This method can be used to assay nearly every

10   component in whole blood. For example, the lipid fractions of the blood can be

individually measured. A good method was a common reagent membrane for

total cholesterol determination in combination with separation layers specific to

the particular fraction of interest.

15   In addition, the present invention, in one form thereof, comprises a

reflectance photometer, which utilizes test strips that are color coded for test

differentiation, thereby making complicated tests less prone to human error. For

example, a blue strip may indicate a glucose test, whereas a red strip may

indicate a cholesterol test. These colors are then divided into shades such as 64

20   shades of blue equal to 64 lot numbers of glucose strips. The photometer

includes a separate optical read head that determines the color and shade of the

base of the test strip device as the strip is inserted into the photometric

instrument. The shade is converted into a lot number ranging from 1 to 64. The

instrument also has a memory module (preferably an electrically erasable

25   programmable read-only memory) that has a corresponding lot number to the

shade of the strip to ensure lot number verification. The instrument then compares the inserted memory module programmed lot number to ensure that it is the same lot number as the test strip. If the strip lot number does not match the memory module lot number, the test is not performed, and the user is

5   instructed to insert the correct memory module.

The lot number verification allows for the automated coding of lot numbers so that the user does not need to enter a lot code for each vial of strips. This prevents the running of the incorrect, old, or expired lot number tests in the instrument.

10   The "plug-in-memory" of the module includes the lot number of the test strip, the expiration date, and the performance criteria for the actual strip measurement. The performance criteria include the wavelength, measurement algorithm, and unreacted density qualifications necessary for a valid test result.

The optoelectronic measurements of the chemistry test reaction on and in

15   a surface enhances the dynamic range of the dry phase test. Algorithms that read at different wavelengths at different times in the chemistry reaction can extend the dynamic range of the test system. This is particularly applicable when using multiple chromophores in a single measurement system. The early portion of a chemistry could be read at the peak wavelength of a reaction, while the later

20   portion, or darker or more dense portion of color development could be read at a wavelength not near the peak of the color development. In addition, different chromophores may respond in a more linear manner in different portions of the dynamic range of the chemistry. Manipulation of these two data points can significantly increase the dynamic range (in mg/dl) of a chemistry reaction.

The optoelectronic measurement of the chemistry test reaction on and in a surface reduces errors due to orientation of the surface to be read to the instrument. Multiple wavelengths and different angles are used to correct possible problems in positioning the strip in the instrument. If the detector is at

5   "0" angle and the emitters of the same or different wavelengths are at different angles (e.g., one at 40° and one at 50°), the tilting of a surface will positively contribute to one reading while it will contribute in a negative manner to the other reading, thus it is able to cancel the error presented by the angle presentation of the surface. These same measurement methods can be used to eliminate

10   interferences from substances such as billirubin and others.

The optoelectronic measurements of the chemistry test reaction on and in the surface enhance the stability of timed and untimed dry phase chemistry reaction. Algorithms are used to determine the "end point" of a chemistry. In other words, measurements can be done at similar or dissimilar wavelengths to

15   predict the stable portion or end point of a chemistry if kinetic measurements are made, the kinetic readings can be subjected to an algorithm to determine that the rate is slow enough to declare the extrapolate chemistry is at an end or completion. When known standards are run and predicted by this pseudo-endpoint, the same measuring criteria can be applied to unknowns to determine

20   the "endpoint' of the test reaction.

The use of colored or shaded visual indicators in the instrument enhance the interpretation of test results. A colored bar graph is used to aid the user in knowing when the user test results are in a normal or safe range. Out of range colors such as orange for caution and red for danger are used when results are

25   outside the green "safe" range. This is particularly useful to new testers who are

not familiar with the number scale of the different test results. A voice module can also be used to warn the user of unsafe results or operation of the instrument system to make the system usable by the visually impaired by providing, for example, a sound beep for each unit of glucose during a glucose test.

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BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view of the reflectance photometer in accordance with an embodiment of the present invention;

Fig 2 is an exploded perspective view of the plastic test strip of present invention

10   in its unlocked position;

Fig 3 is a perspective view of the plastic strip of Fig. 2 in its locked position; and

Fig 4 is a sectional view of the plastic strip;

Fig. 5 is a block diagram schematic of one embodiment of the reflectance photometer of the present invention;

15   Fig. 6 is a graph plotting sample size, elapsed test time and percentage of reflectance illustrating how endpoint determinations may be utilized to speed chemistry measurement.

BRIEF DESCRIPTION OF THE PREFERRED EMBODIMENT

20   In accordance with the embodiment of the present invention, the diagnostic chemistry measurement device 10 for dry solid phase chemical, enzymatic, immunological assay of whole blood or sera analytes is made up of an injection molded carrier test strip 20 in which several porous and nonporous materials containing chemicals and reactants are contained for the purpose of

25   generating a detectable signal in the presence of certain analytes. The test strip

12 is inserted into a reflectance photometer. The reaction material layer and the

test strip 12 is held in intimate noncompressed contact with a whole blood

separation layer in the absence of adhesives for the purpose of providing a liquid

sample free of red blood cells to the reaction layer or layers.

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HOLDER

The holder test strip 12 of this invention acts as holder for the different

layers of the test reaction system. It provides a convenient handle as well as a

mechanism for placing test strip 12 into an instrument 10 for the reading of the

10   density changes of the reaction layers. As shown in Fig. 2 test strip 12 includes

an elongate body 16 preferably formed by injection molding . Elongated body 16

includes a first end portion 18 and a second end portion 20. A hinged portion 22

is located between first and second and end portions 18 and 20 so that first end

18 is foldable over elongated body 16 into contact with second end 20.

15   As shown in Fig. 2 first end portion 18 includes an opening 24 while

second end portion 20 includes a complementary spaced opening 26. When first

end portion 18 is folded over body 16, each opening 24 and 26 are aligned. In its

folded position as shown in Fig. 3 opening 24 in test strip 12 defines an area for

depositing a body fluid sample while opening 26 defines an area in which

20   optoelectronic measurements of chemistry test reactions are conducted.

Test strip 12 further includes a non-adhesive carrier layer 14 formed from, for

example, five particular layers. In a standard diagnostic test strip, carrier layer 14

may include a disbursement layer 28, formed of, for example, woven materials

such as polyester or cotton, for rapid and even disbursement of body fluid along

25   carrier layer 14. Beneath that may be included a separating binding and/or

precipitating layer 30, constructed of known materials such as shown in Table IX

infra, that, when exposed to a sample liquid, may separate analyte and analyte

disrupting elements such as red blood cells from whole blood. This action would

permit the serum analytes to pass through separating layer 30a and 30b while

5   preventing red blood cells or other analyte disrupting elements from passing

through. The last layer shown in Fig. 2 is that of the test reaction membrane 32

on which the dry chemicals and reactants are contained for generating a visible

signal, in the presence serum analytes. Molded carrier body 16 serves as a

support for the reacting and non-reacting layers 28, 30 and 32 which may be

10   formed from papers, membranes and deles materials.

The test strip holder 12 positions the different layer materials 28, 31, 32

within the holder the correct X, Y and Z axis positions. Carrier layer 14 made up,

for example, the disbursement separating and test reaction layers 28, 30 and 32

are held in noncompressed non-adhesive locations by first end portion 18 folding

15   over to second end portion 20. This may be accomplished in a number of

different ways. The preferred way of noncompressingly holding carrier layer is of

an upstanding annular rim 34 may help locate the carrier layer 14 within test strip

12. Additionally, small upstanding protuberances 36 along second end portion 20

radialy located away from opening 26 prevent movement of carrier layer 14. The

20   purpose of both annular rim 34 and small upstanding protuberances 36 is to hold

the layers of carrier layer 14 without compression between opening 24 and

opening 26, hereby preventing pooling of any sample within carrier layer 14. This

consideration of noncompression of the carrier layer 14 is of greater importance

when larger numbers of layers are utilized. The positioning of a carrier layer 14

25   without adhesives or compression allows for efficient transport of sample and

reactants contained in the system and test strip 12. Annular rim 34 or alternatively other areas of test strip 12 may include sawtooth protrusions to increase flow rate thorough carrier layer 14.

Test strip 12 includes a locking mechanism to prevent any unlocking of
5   front-end portion 18 from its folded position over elongated body 16. As shown in Fig. 2, one type of locking mechanism may include a plurality of upwardly extending tabs or projections 38 that interfit or lock into corresponding openings 40 in first end portion 18. When first end portion 18 is folded to second end portion 20, lock projections 38 will interfit and snap lock with openings 40. Other
10   types of one way locking mechanisms may also be used such as snap rivets.

More than one test reaction system can be housed in a test strip 12. A second set of holes 24, and 26 may be included in test strip 12 so that two tests may be run at once.

The described holding mechanism allows for the rapid separation of whole
15   blood into its liquid and solid components. It also allows sample volumes as low 2.0 microliters to be used in dry phase chemistry reactions. Test strip 12 allows the use of several reaction and non-reaction layers. A typical holder could contain from 1 to 8 layers of material with thicknesses from approximately 0.002 inches to 0.007 inches, for example.
20   Chemicals and materials are employed to allow for the treatment of samples such as whole blood, which will allow the whole blood sample to be separated without disrupting the red blood cells while rapidly moving the liquid portion of the whole blood sample to one or more reaction sites in the holder, normally on a test reaction membrane 32. These chemicals can be composed of polymeric and
25   nonpolymeric substances that are dried onto one or more surfaces of the

materials contained in the device holder. Additionally, light metal salts of

elements such as Potassium, Lithium, Sodium, and Calcium may be utilized to

treat red blood cells before and during the separation process. The materials

which may be used in the holder for treatment by or containment of these

5   chemicals can be composed of woven, nonwoven, napped or flocked materials.

LDL

The present invention includes a homogeneous dry phase assay for LDL

Cholesterol that can replace assays that previously required more than one

10   operation to achieve the desired measurement. Enzymes are used to digest HDL

Cholesterol. This method uses a chemical complexing layer (ex. Surfynol 465,

Surfynol 1485, Silwet L 7600, nDodecyl-B-D maltoside, Polyoxyethyglene ether,

Sucrosenomocaphate. A hydrophobic barrier does not allow the complex formed

in #2 to react with the cholesterol reaction membrane. The best example of this

15   is a Polyether sulphane asymmetric, treated with casein, to make it hydrophobic.

Casein coated membrane will not function alone because of its hydrophobicity:

this property is adjusted by the addition of sorbitol and other agents, such as

sucrose or tween 20. In comparison to PCT #WO95/16207, a barrier to control

flow through the device is used, in contrast to the devices described in this

20   publication. This device does not have an absorbent or wick at far end of device,

and this is not a chromatographic device. It was found that when the flow of

liquid into the capillaries of a dry device encounters a restrictive barrier, the

change in pressure due to this resistance can accelerate in one direction only

until the device (the final reaction membrane) is wetted by the sample.

11   12

Thus, the sample flows in one direction in a more rapid rate than any other

direction. This unidirectional flow allows for the chromogen and other reactants

to move in one direction only without the use of an absorbent as in WO95/16207.

Thus, the flow rate is controlled by the use of multiple holes, dissimilar in size,

5   (not unlike adjusting a hose sprayer nozzle.) Examples include: aperture sizes

for aperture A, 0.050-0.250 and aperture B, 0.2-2.0 microns, and for Aperture C,

whatever test strip holder size is. The apertures control unidirectional flow

without using a proximal (final) absorbent layer (wick). Other devices with

apertures contain a wick downstream. This construct is also used for HDL.

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HDL

The present invention includes a single reaction membrane with low

holdup volume for the precipitation and/or binding of VLDL and LDL. This makes

it possible to rapidly detect HDL in one step using whole blood. This direct flow-

15   through device is different than devices from BMC, Kingston and Cholestech that

are all tangential flow systems.

Use of CHAPS in place of cholate in cholesterol reagent

CHAPS, or 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate, is

20   made by Research Organics of Cleveland, Ohio, for use in cholesterol reagents

because of problems people were having with cholate, the usual surfactant is

diagnostic cholesterol reagents. The following are some advantages of using

CHAPS in place of cholate in the reagent:

12

1. CHAPS is more soluble and also more easily reconstituted by plasma than is cholate, thus it can more readily enhance pancreatic esterase activity than cholate.

2. As a result of the increased pancreatic esterase activity there is more chromogen production and thus the resolution and dynamic range of the test is increased.

3. Since CHAPS is more readily resolubilized than cholate, the diagnostic test strips will have greater precision with CHAPS because it will be maximally and more uniformly distributed throughout the reaction membrane immediately after the addition of blood to the strip.

4. Because of its different properties, CHAPS was found to be more compatible with a larger number of different chromogenic systems, particularly the Trinder systems than was cholate. In solutions made with cholate, 4-aminoantipyrine could not remain in solution.

5. Since CHAPS is a more acidic surfactant than cholate, it is able to go into and remain in solution at a lower pH than can cholate. This allows for lowering the pH of its reagent from 6.8 to 6.0 and thus eliminating uric acid interference.

Use of MES buffer in cholesterol reagent

When MES, or 2-[N-morpholino]ethanesulfonic acid, was used as a buffer in cholesterol reagent in place of citrate, it was found to:

1. help solubilize and stabilize (keep in solution) certain cholesterol esterases necessary for the optimal performance of the assay.

2. Increase the amount of color generated per mg/dL of cholesterol thus improving the test's precision. This is probably the result of better performance by the esterase(s).

Substitution of Rhodasurf ON-870 for Triton X-100 in a strip based D-3-hydroxybutyric acid assay

When Rhodasurf ON-870 (a mixture of ethoxylated tridecanol, ethoxylated

5   tridecanol phosphate ester, and phosphoric acid), or a similarly formulated

surfactant, was used in place of Triton X-100 (t-Octyphenoxypolethoxyethanol),

or a similarly formulated surfactant, in a dry, strip based D-3-hydroxybutyric acid

assay the following benefits resulted:

More ready solubilization and hence reconstitution of the tetrazolium indicator

10   resulting in:

1. Increased precision due to more uniform dispersion of regents after

reconstitution with whole blood;

, 2. More rapid kinetics, resulting in the average test time dropping from 120"

to 40"; and

15   3. Increased dynamic range; from 0-10 mg/dL to 0-60 mg/dL D-3-

hydroxybutyric acid.

Enhanced stability of the D-3-hydroxybutyrate dehydrogenase.

The first benefit, as well as the three numbered entries that it subsumes, is

probably applicable to a number of assays using tetrazolium salts as a

20   chromogen, especially in a dry format.

ANALYTES

A wide variety of analytes can be determined by using the disclosed

apparatus. Examples are given in tables I and II, infra.

Further, given the small size and robust nature of the reagent strips and instrumentation, analyses need not be limited to traditional clinical laboratory settings. The device of the present invention is also simple enough to be used by

5   people with minimal or no chemical or medical technology training. This advantage allows use at home, or by mobile health are delivery people. Examples of this are diabetics who must monitor themselves for glucose and ketone bodies, patients on home dialysis who would benefit by monitoring of urea nitrogen and people endeavoring to lower their cholesterol levels.

Further, by combining several different reagents on a single support, a

10   panel of tests may be done. Examples of this would be a liver panel consisting of ALT, AST, Alkaline Phosphates. A diabetic panel might consist of glucose, beta hydroxybutryrate and glycated hemoglobin. A coagulation panel might consist of Prothrombin time, ACTT and ACT.

15   FAMILIES OF ANALYTES BY STRUCTURE

Table 1

Family	Examples
Carbohydrate	Glucose, lactose, galactose
Nitrogen Moiety	Urea nitrogen, creatnine, uric acid
Lipid	Cholesterol, triglycerides, LDL, HDL
Enzyme	ALT, AST, Alkaline Phosphatase, CPK, CK-MB
Hormone	Hcg, lh
Therapeutic Drugs	Theophylline
Drugs of abuse	Cocaine, marijuana, barbiturates, salicylates
Electrolyte	$Na + K + C1 Li + CO_2$

Nucleic Acids	Infectious disease, forensic applications, genetic disorders

FAMILIES OF ANALYTES BY DISEASE

Table II

Disease	Examples
Diabetes	Glucose, beta hydroxybutyrate, hemoglobin A_{1c}
Liver problems	ALT, AST, bilirubin
Acidosis/Alkalosi	PO_2, $PCO^2 1$ pH
Hypertension	Na+, K+
Nutritional status	Ca, Mg Zn trace minerals

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Examples

The following illustrative examples teach various combinations of buffers, dyes, stabilizers and other reactive and functional components which may be combined by a person having ordinary skill in the art into the system test reaction

10   areas.

Table IX gives various types of dyes and indicators used in diagnostic reagents.

Example #1

Glucose measuring system

15   Table III

Ingredient	Function	Amount	Available from:
Glucose Oxidase	Reactant	25,000	Sigma Chemicals

16

			St Louis, MO
Peroxidase	Reactant	75,000	Sigma Chemicals St. Louis, MO
Silwet 7500	Serfactant	0.10 ml	Dow-Corning Midland, MI
PVP K 30	Enzyme Stabilizer	0.50 gms	ISP Linden, JN
Citric Acid	Buffer System	1.25 gms	Aldrich Chemical Milwaukee, WI
Sodium citrate	Buffer System	0.10 ml	Dow-Corning Midland, MI
DOW 1520	Antifoam	1.00 gms	Aldrich Chemical Milwaukee, WI
4 AAP	Chromophore	0.25 gms	Aldrich Chemical Milwaukee, WI
3 5 DCHBS	Chromophore	0.25 mgs	Boehringer Mannheim
Distilled H_2O	Solvent	QS to 100 ml	

Preparation: Approximately 50 ml of distilled H_2O was placed in a beaker on a stirring plate. A magnetic bar was added and the ingredients added sequentially after the previous gradient was dissolved and dispersed. After all ingredients

5 were added the volume was adjusted to 100 ml of distilled H_2O .

Example #2

Triglycerides measuring system

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TRIGLYCERIDES + H_2O $^{CHOLESETEROL\ ESTERASE}$ GLYCEROL + FREE FATTY

ACIDS GLYCEROL + ATP $^{GLYCEROL\ KINASE}$ L – ALPHA – GLYCEROPHOSPHATE

+ H_2O_2 H_2O^2 + 4-AMINOANTIPYRINE + DCHBS ^{PEROXIDASE} QUINONEIMINE

CHROMOPHORE

5   Table IV

Ingredient	Function	Amount	Available from
Cholesterol esterase	Reactant	15,000 units	Shinko-American NY, NY
Glycerol kinase	Reactant	5,000 units	Shinko-American NY, NY
Glycerophosphate Oxidase	Reactant	5,000 units	Shinko-American NY, NY
Peroxidase	Reactant	5,000 units	Shinko-American NY, NY
4 AAP	Chromogen	1.0 ogm	Aldrich
3, 5 DCHBS	Chromogen	0.25 gm	Boehringer Mannheim
MES	Buffer	2.50 gm	Research Organics
PVP K30	Stabilizer	0.50 gm	ISP
Glucose	Filler	2.50 gm	Sigma
Triton X-100	Surfacant	0.10 gm	Boehringer Mannheim
Distilled H_2O	Solvent	QS to 100 ml	

Preparation: Same as example #1

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Example 3

Cholesterol measuring system (all amounts approximate)

Table V

Ingredient	Function	Amount	Available from
Choleseterol oxidase	Reactant	10,000	Shinko-American NY, NY
Cholesterol esterase	Reactant	7,000	Shinko-American NY, NY
Sodium phosphate 0.5 M pH 7.0	Buffer	750 ml	Dow-Corning
BSA	Surfacant	15 gm	Aldrich Chemical
Peroxidase	Reactant	170,000	Shinko-American NY, NY
DOSS	Surfactant	7.0 gms	Boehringer Mannheim
Sucrose	Stabilizer	1.0 gms	Sigma Chemicals
TMB	Chromogen	10.0 gms	Aldrich Chemical
Distilled H_2O	Solvent	QS to 100 ml	

5   Preparation: Same as example #1

Alternatively, the chromogen may be prepared in an organic solvent matrix

and treated as a first or second application to the membrane or paper.

Table VI

Ingredient	Function	Amount	Available from
Acetone/methanol	Solvent	100 ml	Aldrich

19

1:1			
Tetramethyl benezidine	Solvent	1.0 ogm	Biosynth Inc Chicago, IL

Example 4

Blood Urea Nitrogen Measuring System

Table VII

Ingredient	Function
Urease	Reactant
H_2O	Solvent
Bcomthymol blue	Chromogen
PVP K90	Film former
Fructose	filler

5

Preparation: Same as experiment #1

Table VII

Types of Indicators

Chromogenic substrate

10 Redox

Leuco dyes

Oxidative couplers

Benzidene Derivatives

Fluorescent labels

15 Dye releasing system

Table IX

20

Separation mechanisms used in dry reagents

Chemical	Physical	Mechanical
Dextran	Hydrophilic polymers	Centrifuge
Sugars	Porous latex films	Filters
Lectin	Polymer & swelling agent	Filters & pressure
Amino acids PEG/polyacrylate	Membranes microfiber cloth	Membranes & differential pressure wedge shape
Thrombin	Napped cloth	Gels
Sintered porous matrix	Coagulants	Density gradient
Agglutinating agents	Glass fibers	
Amine polymers	Hollow fibers	
Trivalent cations	membrane	

Example 5

5   HDL Formulation

Table X

Chemical	Quantity/100ml
DI WATER	80 g
PTA	1.70 g
Mg Sulfate	0.75 g
Sodium EDTA	0.10 g
DiPotassium Phosphate	1.21 g
QS to 100 ml	

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Example 6

LDL Formulation

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Micell layer (i.e, Michele layer) S&S 595 coated with Surfynol (.9%); Alpha-Cyclodextrin (1.5%) Tween 20 (0.05%); BSA (1%); NaCL (1%); d-Sorbitol (1%). Separation Layer, BTS#45, BTS#5, or Millipore PES tight side down, coated with Casein (Pierce Blocking Solution) and 1%D-Sorbitol. Standard Cholesterol

5   Membrane, Biodyne A.

SPECTROPHOTOMETER

The present invention also includes use of a spectrophotometric device 10

10   for determining the density of the color reaction on and in the membrane surface of the test reaction layer 32 within test strip 12. Photometric device 10 as shown in Fig. 1 includes a hand-held housing 50 for containing electronic control circuitry for operation the aforementioned tests. In the embodiment shown in Fig. 1, a test strip holding region 52 is located above three light detectors or sensors 54 each

15   disposed within a port 56. During test operation, a test strip 12 is inserted into holding region 52 so that test strip openings 26 are located adjacent ports 56. Light sensors may take a reading from light reflected from the exposed test reaction membrane layer 32 or from test strip 12 itself to determine its color.

Housing 50 further includes a specialized display device, such as a liquid

20   crystal display 58. Display 58 is utilized for relating test results and other information to the user. In particular, a color scale 60 is used to facilitate interpretation of test results operating concurrently with digital display segments 62. Additional display segments on display 58 include a test wait indicator segment 64 to inform the user to wait while device 10 is performing the selected

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tests, and a test name segment 66 which the unit determined from the type of test strip 12 inserted.

Color scale 60 may easily be constructed by a plurality of shaded or colored segments arranged adjacent each other to form a bar graph like

5   indicator. Electrically controllable segments 68 are oriented over the color or shaded segments so that when segments 68 are activated segments 68 become dark, preventing certain colored or shaded segments 60 from being visualized or viewed. Segments 68 that are not activated permit the underlying colored or shaded segments of color scale 60 to be visualized. In this way it is possible for

10   an electronic control to permit only a single colored or shaded segment to be viewed thereby communicating test results.

A possible result range spectrum for color scale indication segments may include particular colors with particular test result meanings such as:

Very high result danger, RED

15   High result danger, RED

High result caution, YELLOW

High result caution, YELLOW

High normal result, GREEN

Normal result, GREEN

20   Normal result, GREEN

Low normal result, GREEN

Low result caution, YELLOW

Low result caution, YELLOW

Very low result danger, RED

Color scale 60 permits an unsophisticated user to instantly visually
5   determine, in one embodiment, if a test result is normal (a green segment
visualized), slightly abnormal (a yellow segment visualized) or dangerous high or
low result (a red segment visualized). Alternatively, if a color liquid crystal display
is utilized, the electronic control for test unit 10 may directly indicate a colored
segment, rather than covering all but one colored segment.

10   A suitable instrument, such as a diffuse reflectance spectrophotometer 10
with appropriate software, can be made to automatically read reflectance at
certain points in time, calculate the rate of reflectance change, and by using
calibration factors and software, output the level of analyte in the fluid tested.
The electronic control mechanism of photometric unit 10 is shown in schematic
15   form in Fig. 5. One or more light sources 70, for example high intensity light
emitting diodes (LED) are disposed in housing 50 to illuminate test strip 12 as
shown by arrows 72. A light detector or sensor 54 can be adapted to generate or
respond to particular wavelengths of light.

Sensor 70 transmits a signal to an amplifier 74 as is known in the art. One
20   type of amplifier available for use is, for example, a linear integrated circuit, which
converts the phototransistor current to a voltage signal.

Appropriate electronic circuitry is utilized to take the output of amplifier 74,
normally a sample and hold unit 76, and transfer the signal to an analog-to-digital
converter78. Analog-to-digital converter takes the analog voltage output from the
25   sample and hold unit 76 and converts it to, for example, a 16 bit binary digital

24

number upon command of a microprocessor/microcontroller unit 80. Preferably an electronic microprocessor/microcontroller 80 utilizing digital integrated circuitry is used to time selected tests, read signals and together with associated programs and data memory 82, calculate and store reflectivity valves and

5   calculate analyte levels from the stored data.

Additional information for particular tests may be stored in a removable EEPROM unit 84 operably connected to microprocessor/microcontroller 80. EEPROM unit 84 is an interchangeable plug-in memory module containing measurement parameters, software, calibration data, and reagent recognition

10   data for particular test strips 12. Additionally, EEPROM unit 84 contains the shelf life data and identity verification information for particular production runs or lots of test strips 12.

,   Automated lot coding is done by color coding the plastic material used to make the test strip holder 12. The color used in test strip holder 12 preferably

15   has 16 different densities that can be distinguished by at least one of the wavelengths used in the optical sensor head 54 of instrument 1.

As the strips 12 are inserted into device 10, the instrument detects a change in the measurement area. This change indicates that a strip 12 has been

20   inserted into the instrument 10. As the instrument detects the insertion of a test strip 12, it reads the densities of at least one of the LED's and calculates the lot number by the above table. Instrument 10 then goes to the EEPROM port connected to microprocessor/microcontroller 80 which has an EEPROM unit 84 inserted. Instrument 10 checks to see that the EEPROM preselected lot number

25   is the same as lot number of test strip 12 that had been inserted into the

instrument. If the lot numbers are the same for test strip 12 and EEPROM 84, the instrument downloads the information contained in the EEPROM and proceeds with the test analysis. The instrument 10 reads the density of the unreacted strip to assure quality of the strip before the test is initiated, if quality is

5   passed then the instrument instructs the user to apply a sample.

A sample is then applied and instrument 10 begins a measurement cycle to ensure that the proper amount of sample was applied to the test strip. When the instrument has determined that enough sample has been applied, it then goes into another cycle to measure the end of the chemistry reaction. When the

10   end of the chemistry reaction has occurred, then the instrument measures the final density and compares it to a measurement algorithm stored in EEPROM unit 84. This measurement algorithm then determines the concentration of the test to be measured by comparing the measured density (darkness) of the color formed and comparing this density number to a table of values through the use of an

15   algorithm stored in the EEPROM unit 84.

After a particular test strip is selected and placed in the unit, a sample normally a whole blood sample from a fingertip or from a pipiter tip (which could have gotten its sample from a tube of blood as in a laboratory type situation), is applied to the sample application spot, opening 24, on test strip 12. A

20   disbursement layer 28 causes the sample to quickly spread over the entire area of carrier layer 14. The separation layer 30 of the test strip spot is allowed to separate out the solids (red blood cells and other analytes disrupting elements) from the liquid (plasma or sera or other analyte containing portion). The separated fluid, i.e., the plasma, sera, or other analyte containing portions, moves

25   to the test reaction membrane layer 32 below the separation membrane 30. The

above fluid migration causes the reactants (analytes such as glucose) in the sample to come into contact with the reactants in test reaction membrane layer 32.

Analytes/fluid contacts reagent layer reaction 32 and initiates an

5 appearance or disappearance of color, depending on its particular reaction. The above presentation of analyte to the reaction layer 32 causes the desired reaction to occur. This reaction causes a color change that can be detected both visually and by the instrument. The color change is then converted into a digital result on the instrument LCD as described above. A comparison color chart can be used

10 to visually determine a reaction quantity scale as in litmus paper.

Instrument 10 can use different wavelengths at different density portions of the reaction to maximize the dynamic range of the chemistry and the limits of the instrument at a particular wavelength.

The "end-point" of the reaction is defined as a point where there appears

15 to be no change or a very small change in density. That is, the chemistry changes color proportional to the concentration of the reactance that has come into contact with the reactance materials in the test pad (membrane). This small amount of change can be a change per time period. An example would be as per the graph in Fig. 6. Detailed information used to generate this graph is that the

20 changes per 5-second time period during the beginning of the test reaction would be greater than 5% reflectance per 5-second time period. When this change is less than 1% reflectance per time period it can be said that the reaction is complete or at an endpoint. The instrument stores this percentage reflectance at this time and uses it as above to determine the concentration of the analyte

25 tested for in the test strip.

The Kubelka-Monk equation of K/S=(1-reflectance)^2 divided by (2 x reflectance) can be used to linearize the percentage reflectance values. This linearization simplifies the algorithm necessary to calculate results. This pseudo endpoint chemistry allows a more stable read time, which in turn allows for a

5 more reproducible answer. Pseudo endpoints also permit a more rapid assay to be performed. Certain other glucose monitoring systems incorporate pre-determined timing circuit. This pseudo endpoint allows for a different method to be used in measuring chemistry reactions, provided one can determine the endpoint of the chemistry by a method other than timing.

10 Multiple wavelengths are used to enhance the dynamic range of chemistry. This is particularly useful when one uses a multiple chromophore indicator system as do some of the above mentioned chemistries. Early portions or low concentrations of a test such as glucose can use a broad range indicator such as TMB to increase sensitivity in the low to mid range of the chemistry.

15 When the test concentration is higher or the reaction faster, a different chromophore is focused upon to determine more dynamic range than the previous chromophore. This allows one to expand the dynamic range by two different methods.

One can also use wavelengths on the peak for more dynamic range and

20 wavelengths off the "peak" absorbence of the test system to enhance or reduce dynamic range and also to enhance or reduce the ""pseudo endpoint" algorithms. Manipulation of these four factors, chromophore A, chromophore B, wavelength 1 and wavelength 2 can allow one to better define the "pseudo endpoint" algorithm and also allow one to optimize the dynamic range of the chemistry which in turn

allows for increased sensitivity throughout the chemistry reaction range with greater precision.

Multiple wavelengths can also be sued with different angles of emission of correct possible problems in positioning the strip in the instrument. If the detector is a "0"

5   angle and the emitters of the same or different wavelengths are at different angles (one at 40° and one at 50°) the tilting of a surface will positively contribute to one reading while the other contributes in a negative manner, thus canceling the error presented by the angle presentation of the surface. These same measurement methods can be used to eliminate interference from substances

10 such as bilirubin and others. When the angle of light incidence is increased from improper positioning of a chemistry read surface to the instrument optics, errors of both gloss and angularity are introduced into the measuring system and can give false low readings.

15 Examples

1. Indicators and chromogens advantageously used in combination
    a. wide range pH test

       Bromothymol blue and methyl red covers pH range of 5 through 9
    b. 4 amino antipyrine + 3.5 dichlorohydroxybenzene sulfonate (4AAP _3.5

20   DCHBS)
    c. TMB + Chromotropic acid
    d. Syringaldazine _ Vanillin Azine

2. Color coding for test and lot identification
    a. Blues, 16 different shades (density)

25 b. Reds, 16 different shades (density)

29

c. Greens, 16 different shades (density)

d. Yellows, 16 different shades (density)

e. Oranges, 16 different shades (density)

f. Browns, 16 different shades (density)

5   g. Magentas, 16 different shades (density)

h. Light blues, 16 different shades (density)

i. Light reds, 16 different shades (density)

j. Light greens, 16 different shades (density)

k. Light browns, 16 different shades (density)

10   l. Light magentas, 16 different shades (density)

m. Cyan, 16 different shades (density)

n. Light cyan, 16 different shades (density)

It will be appreciated that the foregoing is presented by way of illustration only,

15   and not by way of any limitation, and that various alternatives and modifications

may be made to the illustrated embodiment without departing from the spirit and

scope of the invention.